

141 contd.

The segregation of the lymphocyte cells from the peripheral blood thus collected may be fulfilled by a common segregating method for lymphocyte cells such as a discontinuous density gradient centrifugation using sucrose, a lymphocyte separator available on the market or other agents.

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Page 6, please rewrite the paragraph beginning at [0024] as follows:

[0024] The cultivation in the invention can be fulfilled by an ordinary cell cultivating method. For instance, the cultivation may be practiced in a CO₂-incubator. It is desirable to carry out the cultivation at the CO₂-concentration of 1 to 10%, preferably 5%, at temperatures of 30°C to 40°C, preferably about 37°C.

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Page 9, please rewrite the paragraph beginning at [0032] as follows:

[0032] In each tube, 44 ml of culture medium (RPMI1640+7 made by Nikken Bio Medical Laboratory Inc.) containing 35,000 U/ml of IL-2 (made by Cetus Corporation) and 5 ml of human serum were added to 50 ml of culture medium (often abbreviated as "medium") and thoroughly mixed by repeatedly turning the tube upside down to obtain cell suspension. Then, the cell suspension was put by 10 µl into tubes (Product 72,690 imported and sold by K.K. Asist) and mixed with 40 µl of Turk's solution (Made by Muto Kagaku Yakuhin) in each tube. The mixtures thus obtained were applied by 10 µl to a hemocytometer (Product No. 9731 made by Perkin-Elmer Corporation) and measured to count the number of cells under a microscope (Model 211320 made by Olympus Optical Co., Ltd.) There were obtained the results that the total numbers of the cells in the tubes were in the range of 1.0×10^7 to 7.0×10^7 .

Page 9, please rewrite the paragraph beginning at [0033] as follows:

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[0033] A solution consisting of 5 μ l of OKT3 (imported and sold by Janssen-Kyowa Co., Ltd. and produced by Ortho Pharmaceutical) prepared previously with 8 μ l of PBS(-) was poured by 10 μ l into a cultivating flask having the base area of 225 cm² (MS-2080R made by Sumitomo Bakelite Company Ltd.) so as to uniformly soak the bottom of the flask in the solution. OKT3 in the flask was sucked out by an evacuator on the next day. Then, upon pouring 50 ml of PSB(-) into the flask, the flask kept covered was vehemently shaken, and thereafter, opened to take out the solution. Again, 50 ml of OKT3 was added into the flask, and then, upon covering the flask with the lid, the flask was vehemently shaken. Thereafter, the flask was opened and remaining liquid contents were courteously removed from the flask and lid, thus to prepare a flask containing solid-phase OKT3.

Page 10, please rewrite the paragraph beginning at [0034] as follows:

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20970-0344650
[0034] To the flask containing solid-phase OKT3 prepared in the "flask arrangement" process described above, 50 ml of cell suspension obtained in the aforesaid "lymphocyte segregation" process was distributed. Then, cultivation in the flask was performed at 37°C in the presence of carbon dioxide gas having a concentration of 5%. After five days, 50 ml of culture medium was added, and the cultivation was continued at 37°C in the presence of carbon dioxide gas having a concentration of 5%. After the following four days, 150 ml of culture medium was added, and further cultivation was performed at 37°C in the presence of carbon dioxide gas having a concentration of 5%. For two more days, the cultivation was continued at 37°C in the presence of carbon dioxide gas having a concentration of 5%. Consequently, 2.0×10^8 to 7.0×10^8 of activated lymphocytes could be obtained.

Page 12, please rewrite the paragraph beginning at [0038] as follows:

[0038] To the aforesaid cell pellets, 200 ml of physiological saline solution containing 2% of human albumin were added to allow the cell pellets to be suspended therein. Lastly, the desired preparations to be administered to a cancer patient were prepared by filtrating the solution through a stainless wire filter of 100 μ in mesh and packing it into a blood transfusion bag. In this case, the number of cells packed in the blood transfusion bag was 6×10^9 to 20×10^9 .

Page 13, please rewrite the paragraph beginning at [0041] as follows:

[0041] Next, one example of cryopreserving the activated lymphocytes prepared in the aforementioned "production" process will be described. Upon centrifuging the activated lymphocytes obtained in the "production" process, the culture medium is removed by decantation to obtain cell pellets. To the cell pellets, 18 ml of cell preserving solution (prepared by mixing 5 ml of human serum, dimethyl sulfoxide (made by Nacalai Tesque, Inc., hereinafter abbreviated as "DMSO") with 40 ml of culture medium (RPMI1640+7)) is added. After fully mixing the mixture thus obtained, it is distributed by 3 ml into five cell preserving tubes of 5 ml in capacity (imported and sold by Corning International) (5×10^7 per tube). The cell preserving tubes thus prepared are placed in a superthermal freezer and preserved at -80°C .

IN THE CLAIMS

Kindly amend the claims as follows:

12. (Amended) The method for preventing recurrence of cancer for a long period of time set forth in claim 1, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.